Collagen/Synthetic Polyanions for Encapsulation of Transduced Melanoma Cells

Wen Shao, Elizabeth M. Jaffee*, Drew M. Pardoll*, and Kam.W. Leong

Departments of Biomedical Engineering, and Oncology*, School of Medicine

The Johns Hopkins University, Baltimore, MD 21205

Introduction

Polyelectrolyte complex coacervation is an attractive process for encapsulating cells because of its mild reaction conditions. We have synthesized microcapsules composed of collagen, a popular substrate for cell culture as the internal layer, and a synthetic polyeletrolyte as the external layer that can be optimized for stability and transport properties.

Although native collagen has many positive and negative charges at physiological pH, it has a net charge density that is too low for microcapsule formation. To circumvent this problem, we raised the pKI of collagen by esterifying its carboxylic groups. At physiological pH, this positively charged collagen was then complexed with a terpolymer composed of methacrylic acid, 2-hydroxyethyl methacrylate and methyl methacrylate. By adjusting the composition of the terpolymer, the charge density and hydrophilicity of the polyanion can be adjusted for optimal physicochemical properties.

Granulocyte–macrophage colony stimulating factor (GM-CSF) has been identified as the most potent cytokine in stimulating the anti-tumor immune response (1,2). One of the keys to success in this approach is that the GM-CSF be delivered only to the tumor site to avoid systemic toxicity. Supply of GM-CSF by the genetically altered cells tends to be transient because of eradication of the cells by the immune system. There is reason to believe that a longer supply of the cytokine may potentiate the anti-tumor immunity. The objective of this study is to determine if that prolonged delivery can be achieved by encapsulating GM-CSF–secreting cells in semi-permeable microcapsules.

Experimental

Collagen was esterified by reacting with methanol for three days in the presence of 0.1M HCl. Different compositions of the terpolymer of methacrylic acid, 2-hydroxyethyl methacrylate, and methyl methacrylate were radically polymerized with 1,1'-azobis(cyclohexane carbonitrile) as the initiator. The polyelectrolytes were characterized by NMR and potentiometric titration and their molecular weights were determined by GPC.

Microcapsules were formed by extruding modified collagen phosphate buffered saline (PBS) solution through a polyethylene tubing using a syringe pump into the synthetic polymer PBS solution. To observe the structure of the microcapsules, collagen was labeled with Texas red and the synthetic polyanion with FITC for confocal microscopy.

B16-F10 cells, which were transduced with GM-CSF gene as previously described (2), were cultured at an initial density of 2.3×10^5 cells/ml on 6-well flat culture plates coated with modified collagen, synthetic polymer and modified collagen/synthetic polymer (the plates coated with synthetic polymer and, then, modified collagen) with plain wells as a control. B16-F10 cells were encapsulated at an initial density of 10^4 cell/ml. These encapsulated cells were cultured in RPMI-1640 containing 10% fetal calf serum and 5% CO_2 at 37°C. Cell viability and growth inside the microcapsules were assessed by uptake of fluorescein diacetate, while cell death was visualized by uptake of propidium iodide and trypan blue. The culture media were collected in every 24 hours. Concentration of the secreted GM-CSF in the culture medium was determined by a ^{3}H-thymidine incorporation proliferation assay of NSF-60 cells, the growth of which is dependent on the presence of GM-CSF in a dose-responsive manner (3). Bovine serum albumin and alcohol dehydrogenase were encapsulated in separate experiments for estimation of the transport properties of the microcapsules.

Results and Discussion

The modified collagen exhibited simple base characteristics as analyzed by potentiometric titration. With a net positive charge at neutral pH, collagen complexed with a synthetic polyanion to form stable microcapsules at physiological pH. The number average molecular weights of the synthetic polyanions were approximately one million with a polydispersity around 1.4. The microcapsules remained stable in PBS and the medium used for B16-F10 culture at 37°C for months. Figure 1 shows the strength and flexibility of the capsule when pressed by a blunt 26 gauge needle; the capsule returned to spherical shape when the needle was removed. Confocal microscopic analysis shows that the internal layer of the microcapsules was predominantly composed of collagen. The diffusion experiments showed that BSA (MW=67,000) was completely released in 15 minutes, while less than 10% of alcohol dehydrogenase (MW=150,000) was released in an hour.

The melanoma cells were first cultured on culture plates coated with modified collagen, synthetic polymer, and modified collagen/synthetic polymer to

test their suitability as substrates. Growth of the cells on all the three different surfaces were slightly faster than TCPS control. No significant differences in terms of cell morphology were observed. The concentrations of secreted GM-CSF from the cells cultured on all the three coated surfaces were slight higher than the control as shown in Figure 2. These results indicated that the components of the capsule wall are suitable substrates for this cell type.

The gene transduced melanoma cells were encapsulated at an initial concentration of 10^4 cells/ml. Some of the cells spread out within 72 hours. The cells formed lumps inside capsules (Figure 3A). After two weeks of culture, the cells grew full of most of the capsules. Stained with fluorescein diacetate, the cells from the capsule are viable (Figure 3B). While our previous work showed that microencapsulated fibroblasts would contract the capsules by about 50% (4), no contraction of the microcapsules was observed for these melanoma cells. Functional activity of the cells inside the capsules was clearly indicated by the daily production rate of GM-CSF, which increased with time as shown in Figure 4. We stopped functional study at day 12, because the cells bursted some of the capsules. Previous studies have identified that a threshold GM-CSF production rate of 36 ng/10^6 cells/24 h is required to achieve systemic immunity in cancer vaccine design (1). These preliminary results indicate that this target is attainable with the encapsulated cells.

This study demonstrates the merit of this cell encapsulation system, suggests an alternative mode of cytokine delivery and provides basis for other cell-based artificial organ designs.

References:

1. E.M. Jaffee et al, *Cancer Res.*, **53**, 2221(1993)
2. G. Dranoll et al, *PNAS USA*, **90**, 3539(1993)
3. T. Kitamura et al, *Blood*, **73**:375, 1989
4. W. Shao et al, *The 20th Annual Meeting of the SOCIETY FOR BIOMATERIALS*, Abstract # 316, Boston, 1994

Fig. 1. A blunt 26 gauge needle pressing the capsule formed by complex coacervation of modified collagen and synthetic polyanion in PBS. The capsule was stained with brilliant blue R.

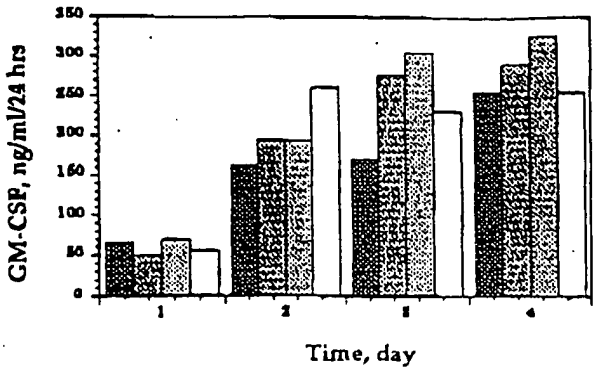

Fig. 2. Secretion of GM-CSF by B16-F10 melanoma cells cultured on different surfaces. Initial cel concentration: $2.3x10^5$ cell/ml. Each 4 columns from dark to light: control, modified collagen, synthetic polymer, and modified collagen/synthetic polymer.

Fig. 3. B16-F10 cells encapsulated in modified collagen and synthetic polyanion. (A) cells grew into lumps one week post-encapsulation; (B) fluorescent photograph of cells in capsule two weeks post-encapsulation and stained with fluorescein diacetate, showing the budding of the capsule by proliferating cells.

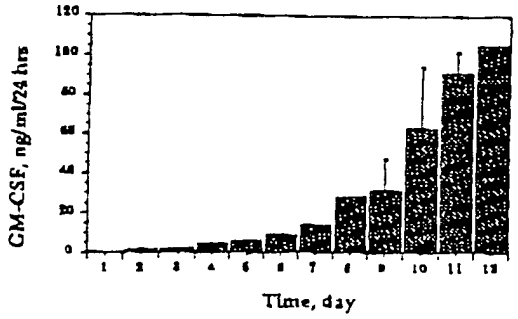

Fig. 4. Daily production rate of GM-CSF by encapsulated melanoma cells. Initial cell concentration: 10^4 cells/ml.